SPECIAL VIRUS - LEUKEMIA PROGRAM

PROGRESS REPORT #4

SPECIAL VIRUS-LEUKEMIA PROGRAM

OVERALL SUMMARY

F. RAUSCHER AND R. REISINGER

The probability is very high that viruses responsible for at least some human and domestic animal leukemias will be found, and that it will be possible to develop measures (including test vaccines) for the prevention or control of these diseases. These predictions could not have been made several years ago and are based largely on recent findings through work conducted within the Special Virus-Leukemia Program (SVLP) of the National Cancer Institute.

A. Background

Beginning in 1964, the Congress of the United States provided funds to the National Cancer Institute (NCI) for an intensified program in virus-leukemia research. These appropriations have been made to support special efforts and to complement the extensive research funded through the usual mechanism of grants in support of work by other investigators.

Utilizing a new planning approach called the Convergence Technique (L. M. Carrese and C. G. Baker, NCI), an overall program plan aimed at controlling human leukemia was formulated. The funding level for this Program in fiscal year 1967 has been approximately $16,500,000. The NCI requests for these supplemental funds have been based on the conviction that there exists sufficient knowledge and information and technical capability to plan and implement an intensified and coordinated Program. The main objectives of this Program are: (1) to determine whether viruses comparable to those now known to be associated with avian and murine leukemia are etiological agents of human leukemia, and (2) to develop an effective vaccine or other means for the prevention and/or control of human leukemia and lymphoma when such etiological agents are found. The main assumption or working hypothesis on which the overall Program is based is that at least one virus is an indispensable element for the induction (directly or indirectly) of at least one kind of human leukemia (including lymphoma) and that the virus and/or virus genome persists in the diseased individual.

From the research standpoint the program is divided into 4 major areas of effort: Human Leukemia Etiology and Prevention, Special Animal Leukemia Ecology Studies, Biohazards Control and Containment, and Human Leukemia Therapy. Approximately 100 of 184 projects, which currently make up the program plan, are being conducted by

investigators in governmental laboratories and clinical facilities, in universities, in nonprofit laboratories, and in commercial facilities. Selection, implementation and continuation of all projects are done on the basis of both scientific excellence and high priority relevance in terms of the integrated program.

B. Scientific Activities - Summary Fy '67

The Special Virus-Leukemia Program was planned and implemented in 1964 and was based on the justifiable conviction that sufficient scientific facts and leads, and, technical competence were available to mount a special, intensive, coordinated program -- a program specifically aimed at the prevention and effective treatment of human leukemia and lymphoma. The validity of this conviction and the definitive scientific base of the program have been significantly strengthened through inhouse (NIH) and contract research development in Fy '67.

The principal laboratories of the Etiology Area, the personnel of which provide the inhouse intellectual and technical motivation, guidance, and thrust for the SVLP are the Branches of Viral Leukemia and Lymphoma, Viral Carcinogenesis, Viral Biology, Epidemiology, and Biometry. In addition, data and guidance from the laboratories of Drs. Robert Huebner, NIAID, Alan Rabson, LCP, John Fahey, LI, Paul Gerber, DBS, and Seymour Perry, Edward Henderson, and Paul Carbone, MB have contributed very significantly to the program.

General. -- Contract projects within this program are chosen and implemented on the basis of technical and intellectual excellence, need, and relevance to specific program objectives. While, therefore, the measurement of virtually all reported data and information are of immediate or predictable relevance, their individual citation and analysis are at this time premature and in this report unnecessary. It is, however, entirely pertinent to discuss the modification and elimination of old and the development of new avenues of approach.

A scoreboard of biologic agents most frequently detected or isolated from human leukemia and lymphoma patients to 1967 would list mycoplasma, unidentified Herpes-type virus, "C" type virus (morphologically identical to avian and murine leukemia viruses), cytomegaloviruses (also Herpes-type) and Reo viruses (particularly type III).

One avenue of approach that has been significantly curtailed concerns studies on mycoplasma as etiologic candidates for human leukemia and lymphoma. In 1966 and through early 1967, investigators of 8

different contract laboratories were heavily committed to mycoplasma studies. While in some cases this appeared to be warranted, a considerable portion of their efforts were being diverted away from virological aspects of the program. The decision to abandon fundamental studies on mycoplasma was based on (a) no apparent differences between the amount and kind of mycoplasma associated with cancer patients and patients with other non-neoplastic diseases, (b) the demonstration that mycoplasma were often present in laboratory passaged specimens as adventitious or inadvertent contaminants derived from aerosols from laboratory animals and people and from trypsin and serums used for tissue cultures, (c) the demonstration that murine leukemia and Rous sarcoma viruses known to be free of PPLO will following inoculation into SPF or germfree mice, also known to be free of PPLO, induce typical progressive leukemia or sarcomas, and (d) the demonstration that virus free of PPLO is recoverable from these induced diseases. Because of these findings and because of their apparent role as ubiquitous contaminants, it was decided to limit work on mycoplasma to the monitoring of leukemic materials which are to be used for virus isolation attempts.

Persistent infections with cytomegaloviruses have been reported in a relatively high percentage of leukemic children and to a lesser extent in leukemic adults. These viruses are usually detected in urine but on few, if any occasion, are they recovered from neoplastic cells or tissue. These viruses have also been detected in a relatively large number of children with other diseases (e.g., infectious mononucleosis) and, of course, in children with cytomegalic inclusion disease.

Reo viruses (particularly type 3) have long been recognized as having an extremely wide spread host range (man, mammals, fish, birds, etc.). British investigators have detected Reo type 3 virus in up to 70% of African Burkitt lymphoma patients. Preliminary studies by Dr. Stanley, Australia, suggest the possibility that Reo virus isolated from a Burkitt lymphoma patient may be capable of inducing lymphomas in a very small percentage of laboratory mice. To our knowledge these reports on the detection of Reo virus in lymphoma patients and the possible induction of lymphoma in a mouse have not been confirmed. Reo virus has not been detected in lymphoma materials derived from African patients and worked up in the United States. Similarly, the virus has not been detected in tissue cultures established from approximately 70 American patients with leukemia or lymphoma. Efforts will, however, be continued within the SVLP to detect and, if warranted, characterize Reo viruses from cancer patients.

The virus or group of viruses most persistently detected and isolated from human leukemia and lymphoma materials is a Herpes-type agent. Morphologically, the particle is identical to known members of the Herpes group including Herpes Simplex, Herpes Zoster, and cytomegalo viruses. This virus(es) appears to be an important candidate for consideration as an etiologic agent of human cancer for the following reasons:

1. Largely within the past two years we have established tissue cultures of cells from the buffy coats and tissues of over 60 patients with malignant disease (predominantly leukemia or lymphoma). Approximately 42 of these lines are known to be infected with a Herpes-type virus. The absence of virus from the remaining lines cannot be definitely established without more exhaustive study because the maturation of the agent is usually observed in a small fraction of the cell population. These lines have been established by 25 investigators in various laboratories and from patients of 5 continents. The different virus isolates appear morphologically identical and at least in some cases appear to share common antigens.

2. This herpes-type virus(es) has not been found to be related to any of the known herpes viruses of animals including man.

3. In addition to its presence in established tissue cultures, HTV has also been found prior to tissue culture in buffy coat and brain cells of human leukemics, and in at least 1 case, also in peripheral leukocytes of the mother of a congenital leukemic.

4. Several of these cell lines have already been grown in industrial quantity wherein it is possible to produce kilogram quantities for subsequent virus extraction. The virus can be recovered, purified and concentrated to the extent that preparations containing approximately 10^{10} to 10^{12} particles per ml are available for study.

5. Herpes virus(es) of identical morphology is associated (perhaps etiologically) with adenocarcinomas of frogs and with lymphomas of the African clawed toad. A cell line started from the peripheral blood of a Rhesus monkey with acute myeloid leukemia also contained a herpes-type virus. More recently, tissue cultures were started from each of two young juvenile chimpanzees following their inoculation with two of the human cell lines known to contain HTV. Both of these chimp lines were heavily infected with a HTV. These findings in chimpanzees may represent (a) the detection of an indigenous chimp virus having morphological and other characteristics in common with human HTV, (b) the transmission of human HTV (for the first time) to an animal recipient, or, less likely (c) survival of HTV containing human cells in peripheral chimp blood.

6. A specific neo-antigen (G) has been found repeatedly to be associated with many human tumors and human cell lines of malignant origin. With only one exception this antigen

had not been found in cells of nonmalignant human tissues or in nonmalignant cell lines. A recent study showed that G-antigen was induced in normal human amnion cells by herpes virus A (a recent human isolate) but not by vaccinia virus. Whether or not a cause and effect relationship exists between herpes infection, appearance of G-antigen, and consequent or subsequent malignancy is not known. In this regard, several other recent studies appear pertinent. The induction of murine papillomata following inoculation of herpes-virus A and 3-methocholanthrene is directly dependent on the amount of infectious virus contained in the synergistic inocula. The survival time of human diploid cells heterotransplanted to the hamster cheek pouch is increased after infection of the cells with several viruses. Six patients who developed recurrent herpetic lesions around the mouth also developed subsequent carcinoma at the sites of the herpetic lesions. If G-antigen is in fact a specific factor related to tumors, these results further strengthen the hypothesis that certain herpes viruses may be oncogenic.

7. The original report by Fink, et al., confirmed by investigators at the University of Michigan and at Roswell Park Memorial Institute, showed that conjugated antisera prepared against pellets of human leukemic plasma known to contain C-type particles could be used to detect and monitor the presence of an immunofluorescent antigen in the buffy coat and bone marrow cells of about 60-70% of patients with leukemia. This immunofluorescent reagent also reacts with the same percentage of cultured cells which by electron microscopy is shown to contain the herpes-type virus. No reaction (fluorescence) is observed with cells infected with herpes simplex, cytomegalo and other human or animal herpes viruses.

The program area in which we have experienced greatest difficulty is that concerned with the detection and replication of C-type particles from human leukemia and lymphoma. Experiences with animal neoplasms indicate that there are at least 22 different viruses etiologically associated with the induction of leukemia and lymphoma in murine, avian, feline, and probably in canine and bovine species. This and other information suggests that if some human leukemia and lymphomas are virus induced, the most likely etiologic agent will be of the C-type. During 1967 we continued to show that the plasmas and in some cases tissues of approximately 30% of human patients contain C-type particles. These particles, however, apparently do not survive or persist in an identifiable form in tissue culture. Conversely, herpes type virus is found seldom, if at all, in plasma but is very frequently found in tissue cultures derived from biopsy tissue and from peripheral leukocytes of cancer patients. While it may be somewhat difficult to understand how virus or virus-like

particles which differ substantially in morphology, site of maturation and presumably in chemical composition can be etiologically related to the same group of diseases (leukemias and lymphomas), a similar phenomonon occurs in animals. Fibrosarcomas of nearly identical gross and microscopic morphology can be induced in hamsters with both polyoma (DNA) and Rous sarcoma (RNA) viruses. It is, of course, entirely possible that the herpes-type virus is an international ubiquitous passenger virus with no etiologic relationship to neoplasia. Until this is determined, however, a large proportion of the resources and effort of the program must be devoted to studies with this virus. The obligation to follow the herpes lead does not mean that efforts with C-type particles will be curtailed. The elusive C-type particle is still considered the prime candidate, and newer leads emerging from model systems will be expeditiously exploited and applied to studies of the human disease.

Major scientific activities which have had to be conducted and accomplished for the prevention and/or control of microbiologically induced diseases are presented in Table 1. These sequential activities appear to be as applicable to the control of human leukemia and lymphoma as they were to the vaccine prevention of smallpox, whooping cough, polio and measles, etc.

Table 1 presents information on the principal model systems available for virological studies on leukemia or lymphoma and attempts to reflect, according to these sequential activities, the current status of efforts towards the control of these diseases in animals as well as in man. Since the discovery by Ellermann and Bang in 1909, that a chicken leukemia was transmissible by an "ultravisible virus" fifty-seven years have elapsed, during which time 3 viruses (or principal groups of viruses) have been found to be associated with avian leukosis. To date no effective method for the prevention or control of leukemia in chickens is available. However during the 15 years since Gross' discovery of the cell-free transmissibility of murine leukemia, approximately 16 viruses have been isolated and several effective ways have been shown for the prevention and control of murine leukemia. Forms of this disease in murine animals can be prevented effectively by vaccination and the disease can be controlled by genetic manipulation of the host to some extent by chemotherapy and by intercepting various forms of horizontal transmission (containment of infected or carrier animals, prevention of feces ingestion, cannibalism, and ingestion of infected milk). A model system which may allow significant studies relevant to the findings of herpes-like viruses in human leukemia and lymphoma cell lines is that reported by Balls, et al. concerning the apparent association of a herpes-like virus with "spontaneous lymphomas" of the African clawed toad (Xenopus species). This animal is common in the Burkitt lymphoma area of Africa and as reported by Haddow, et al., mosquitoes which bite man in that area also, on occasion, feed on toads.

TABLE 1

SPECIAL VIRUS-LEUKEMIA PROGRAM - 1967

ACTIVITY	LEUKEMIA/LYMPHOMA							POLIO	MEASLES
	AVIAN	MURINE	FELINE	CANINE	BOVINE	AMPHIB.	MAN	MAN	
ACQUISITION OF MATERIALS	1909	1951	1961	1965	1960	1965	1957 1963	1900	1930
DETECTION									
ISOLATION						"H"	"C"		
REPLICATION									
IDENTIFICATION									
CHARACTERIZATION									
INDUSTRIAL REPLICATION							"H"		
CONTROL									
ANIMAL:	AVIAN	MURINE	FELINE	CANINE	BOVINE	AMPHIB.	MAN	MAN	
YEARS:	57	15	4	1	6	1	9 3	58	34
NO. "VIRUSES":	3	16	1	-	-	-	-	3	1
DISEASE CONTROL:	None	Vaccine Genetic Therapy Fomite	None	None	Sacrifice	None	Therapy	Vaccines	

In man the beginning of a major effort to detect and characterize viruses from human leukemic materials began in 1957 with Dmochowski's findings of "C" type particles in the lymph nodes of human patients, and, in 1963 following reports by Epstein, et al. and Grace, et al. of herpes-like viruses in cell lines of Burkitt lymphoma and chronic myeloid leukemia patients, respectively. If one assumes that (a) one or both of these virus types are etiologically related to the disease in man, and that (b) reports of studies conducted with these candidate viruses are valid then the overall progress towards sequential step control is, for type "C" particles -- between the steps of isolation and replication; and for the herpes-like particles -- between the steps of characterization and industrial replication.

Table 1 also presents information which compares progress towards the prevention or control of leukemia in animals and man, to non-neoplastic human diseases for which effective vaccines have recently been developed and used. Approximately 58 years elapsed between the demonstration that polio was a virus-induced disease and the development of an effective vaccine for 3 antigenic types of virus. Similarly, it took approximately 34 years to develop an effective vaccine, with 1 virus type, for measles. It is to be noted that similar ongoing studies with "C" type and herpes-like virus particles of human leukemia have been conducted for 9 and 3 years respectively.

Progress Highlights. -- Several of the most significant facts and leads from studies conducted within or related to this program predominantly during Fiscal Year 1967 are as follows:

1. PROGRAM PLANNING AND MANAGEMENT -- A very high effective level has been attained in the coordination of like scientific activities conducted by investigators in Government, university and commercial laboratories and clinics -- and in the identification and integration of different activities relevant to the objective of the total program. Specific decision criteria for each major scientific area have been identified which must be met prior to systematic advancement from one scientific phase into another.

   This is important because they allow, within a program motivated in part by a strong sense of time urgency and for which funds, personnel, and space are not unlimited, clear identification of (a) projects of highest excellence, need and relevance, (b) less fruitful avenues of approach, (c) areas of desirable (and undesirable) duplication, and (d) projects which are scientifically sound but which even if performed successfully would not measurably contribute to satisfying decision criteria and therefore, attainment of overall program objectives.

2. DETECTION AND ISOLATION OF VIRUSES -- It is now apperent that virus-like particles of two different types can be detected, and in some cases isolated, from patients of different countries who are afflicted with all kinds of human leukemia or lymphoma. Particles of one kind ("C" type) are identical with those known to cause leukemia in laboratory animals. Viruses of the other type are similar in size and shape but not identical to those known to cause fever sores or shingles in man, and lymphomas and carcinomas in toads and frogs.

   This is important because the repeated detection and isolation from patients of viruses similar to those known to cause cancer in animals is -- in itself -- indirect suggestive evidence of virus involvement in human cancer, and is prerequisite to the growth of larger quantities of virus, in the laboratory, for studies which may lead to a vaccine.

3. GROWTH OF HUMAN CANCER CELLS IN THE LABORATORY -- SVLP contractors and others have firmly established the growth of tumor cells in the laboratory from 105 different patients with leukemia, lymphoma and other cancers (See Table 2). Eighty of these tissue culture cell lines were derived from children and adults of Africa, Australia, England, Sweden and the United States suffering with leukemia or lymphoma. The remaining 25 tissue cultures were begun from living tumor materials obtained from American patients with other forms of cancer.

   This is important because we can now grow those quantities of living human cancer cells in the laboratory which are necessary for virus isolation attempts. Since these cancer cells reproduce in number for many years, newer techniques of virus detection and isolation can be applied to a standardized source of human material. It is no longer necessary, therefore, to rely completely on the procurement of fresh patient material for each laboratory experiment. This is of paramount importance.

4. RECOVERY OF VIRUS FROM HUMAN LEUKEMIA AND LYMPHOMA TISSUE CULTURES -- Virus particles of identical size and structure have been seen in at least 60 of these human tissue cultures (See Table 2). We can now grow industrial quantities of these tumor cells and recover large quantities of purified and concentrated virus.

TABLE 2 -- SUMMARY: HTV AND HUMAN CELL "LINES"

CATEGORY	PATIENTS CULTURED / PATIENTS ATTEMPTED *		PATIENTS HTV + ** / PATIENTS CULTURED		LINES HTV + ** / LINES CULTURED	
	No.	Percent	No.	Percent	No.	Percent
Lymphoma	31/61	51%	19/31	61%	23/41	56%
Lymphoid Leukemia	15/111	14%	4/15	27%	4/17	24%
Myeloid Leukemia	34/138	25%	14/34	41%	23/100	23%
Sarcoma-Carcinoma	25/54	46%	7/25	28%	11/46	24%
TOTAL (CANCER)	105/364	29%	44/105	42%	61/204	30%
Other diseases	5/54	9%	1/5	20%	1/5	20%
Normal	14/81	17%	3/14	21%	4/20	20%
TOTAL (NON CANCER)	19/135	14%	4/19	21%	5/25	20%

HTV = Herpes type virus; (prototype = Epstein's EBl).

* Approximate. Successful attempts understandably are nearly always reported; nonsuccesses, usually not.

** These numerators are not identical because (a) several positive lines have been started from the same patient and (b) up to 6 lines have been started from the same patient of which only 1 or 2 are positive.

This is important because it provides large quantities of virus from human patient materials which are needed to determine ability to induce cancer in animals, and comparisons of similarities and differences to other human viruses and to animal viruses known to induce cancer in animals.

5 DISTRIBUTION OF CANDIDATE VIRUSES "IN NATURE" -- Herpes-type viruses which appear to occur most frequently in leukemia and lymphoma patients have also been found in horses, frogs, toads, snakes, monkeys, chimpanzees, and in people with non-cancerous diseases as well as in "normal" persons. Viral antibodies have been found in 10-80% (depending on age) of normal people as well as in nearly 100% of persons with certain kinds of cancer. Virus particles have been found in the blood and tissues of very young leukemic children as well as of their parents, brothers and sisters. Herpes-type virus was recently found in blood cells of a child born with leukemia and in blood cells of the normal (non-leukemic) mother.

This is important because it (a) indicates the ubiquitous widespread distribution of this virus in nature, (b) suggests that a "normal" nondiseased carrier may be a source of infection and disease for highly susceptible individuals, (c) suggests similarities to other human diseases such as polio wherein approximately 95% of people were infected although less than .0001% developed paralytic disease, and (d) suggests that viruses associated with human leukemics may be transmitted, before birth, from mother to offspring as are known leukemia viruses of mice and chickens. An understanding of these phenomena is of utmost importance to the feasibility and use of preventative vaccines.

6. DEVELOPMENT OF SYSTEMS TO TEST LARGE NUMBERS OF HUMAN SERUMS -- Reliable and sensitive laboratory methods have been developed which will allow us to determine the number and type of people in selected populations who have been exposed to and possibly infected with these viruses.

This is important since it is not possible to obtain direct proof of the ability of a human virus to induce cancer in man -- i.e., we can not inoculate man deliberately with a suspect tumor virus. We can, however, obtain indirect

evidence or proof of cancer-inducing capability, by determining whether certain cancer patients are infected with the suspect virus in higher numbers, or more severely than normal people or patients with other diseases.

7. RECOVERY OF VIRUS DIRECTLY FROM PATIENTS -- Collaborative studies conducted between laboratory and clinical investigators have made it possible to collect very large fractions of blood suspected of containing leukemia viruses. This is done by separating and retaining the plasma and by returning the essential blood cells to the patient. This has made it possible to detect and recover larger quantities of virus directly from different patients than previously were available.

This is important because it will allow comparative studies of the "C" type particles (which to date can not be grown in the laboratory) recovered from leukemic human plasma with the herpes-like viruses derived from the 60 different cultures of human leukemia or lymphoma cells grown in the laboratory. It will also allow comparative studies of human "C" type particles with similar viruses known to induce leukemia in laboratory and domestic animals.

8. ATTEMPT TO TRANSMIT HUMAN LEUKEMIA TO LABORATORY ANIMALS -- It has now been established that at least one tumor virus known to cause cancer in chickens and other bird species will induce malignant sarcomas in marmosets, a species of small monkeys. Materials from human patients have already been inoculated into 600 newborn monkeys and chimpanzees of various species, and into large numbers of dogs, cats, mice, hamsters, etc.

This is important because the leukemia or tumor-inducing ability of human viruses must be determined in a non-human animal -- preferably one which is phylogenetically close to man. Successful induction of leukemia in animals with a specific human virus will also provide a test system for more effective utilization of drugs and evaluation of procedures for the treatment of human leukemia.

9. EXPANSION OF STUDIES ON AFRICAN AND AMERICAN LYMPHOMAS -- It is now firmly established that while Burkitt lymphoma occurs in African children at an incidence higher than a combination of all other forms of childhood leukemia and lymphoma in Africa, the disease also occurs in a small but significant number of individuals in the United States and other countries. In Africa this disease occurs in children who live predominately in geographical areas characterized by certain temperature, rainfall, and altitude limits. Because of the incidence of this particular tumor, and because some of the characteristics of the disease strongly suggest that an insect vector may be involved in the transmission of the African disease, we have established and are coordinating close working liaisons with key scientists and clinicians in Africa and in the U. S. In order to continue these working relationships and to more effectively feed back information to our African colleagues, a member of our professional staff will spend a year in Africa for the purpose of lecturing to medical students, of obtaining clinical specimens for studies in laboratories of the NCI, and to set up a controlled epidemiological study.

   This is important because it establishes a firm collaborative study with scientists and clinicians knowledgeable about a most unusual childhood lymphoma which currently is considered most likely to be associated with a causative virus. The establishment of an epidemiological survey is of utmost importance because certain regions of the Burkitt lymphoma area in Africa may be used as one of the field trials for testing a candidate vaccine.

10. SOME TUMOR VIRUSES REQUIRE HELPER VIRUSES -- A highly potent mouse sarcoma virus was discovered this year. The major significance of this new laboratory model is the finding that this virus requires the help of a leukemia virus (dual infection) for the induction of malignant sarcomas. These studies parallel those previously reported with several sarcoma viruses of chickens and suggest that the helper phenomenon may contribute to the occurrence of tumors in other animal species -- including man. To test this hypothesis tissue cultures have already been established from 5 human sarcomas, the microscopic type of which is identical to tumors induced in mice with the Moloney sarcoma virus.

This is important because it may open the way for disclosing causative viruses associated with human sarcomas and may provide a new avenue of approach to the search for human leukemia viruses.

11. PRODUCTION OF EFFECTIVE VACCINES FOR MOUSE LEUKEMIA -- It is now recognized that at least 16 virus strains are capable of causing leukemia in mice and rats. It appears, however, that similar to polio in man, these viruses fall into only several different antigenic types. It has already been shown that a vaccine prepared against one particular virus will immunize mice not only against that virus but against others as well. As has recently been accomplished with polio and measles viruses, both live and killed vaccines have been prepared with mouse leukemia viruses. In some cases the vaccines and/or their antibodies together with drugs are reasonably effective when administered to leukemic animals.

This is important because it shows that (a) animal leukemia can in fact, be prevented with vaccines and that the causative viruses from which the vaccines were prepared can be attenuated and/or killed by procedures already established for the influenza, polio, and measles diseases of man, and (b) it is possible to treat (by vaccine or combination immuno-chemotherapy) mice after leukemia develops. This approach may make possible more effective control of the human disease than that attained by drug therapy alone.

12. IMPLEMENTATION OF COLLABORATIVE CONTRACTS TO STUDY LEUKEMIA IN CATTLE, CATS AND DOGS -- New and unique facilities have been constructed and are currently being used to determine the number and type of leukemias and lymphomas in cattle, cats and dogs which are caused by viruses and to determine whether the newborn of these species, as well as monkeys, can be used as sensitive indicator hosts for candidate leukemia viruses recovered from man. We have already succeeded in establishing lines of leukemia cells from cattle and dogs which can be transplanted into these animals with the causation of leukemia. Electron microscopic studies of the transplantable tumor of cattle and of an established tissue culture of a dog lymphoma have revealed the presence of large numbers of type "C" virus particles identical in size and shape to those viruses known to induce leukemia in mice and rats.

These findings are important because they strongly indicate that many of the leukemias and lymphomas which occur in animals that have a prolonged and very close association with man throughout his life are also virus-induced. If this is indeed proven to be true, then means can be implemented for the prevention and/or control of animal leukemias and possibly of some human leukemias. It will be possible to isolate, treat or sacrifice animals known to be carriers of their own leukemia viruses and to improve, if necessary, currently used methods of treating consumable products, such as milk, milk products, and meat.

13. INSECTS AND ANIMAL TUMOR VIRUSES -- It is known that mites are capable of transmitting chicken leukemia viruses, and that certain mosquitoes can transmit avian sarcoma viruses from one chicken to another. Species of kissing bugs, bedbugs and cockroaches are capable of maintaining infectious mouse leukemia virus for periods up to 72 hours. Most recently tumors were induced in fruit flies with the Rous sarcoma virus of chickens. Studies are being conducted to determine whether these cancer viruses actually grow (replicate) in insects or whether the virus simply survives between blood meals and is therefore transmitted through the mechanical process of biting.

These studies are important because they may provide (a) clues on how tumor viruses are transmitted in nature, (b) added credence to the hypothesis that Burkitt lymphoma of African children is insect transmitted, and (c) a means for controlling the incidence of some cancers similar to the control of malaria through eradication of mosquitoes.

14. BIOHAZARDS AND CONTAINMENT -- During the past year it was shown that highly potent laboratory strains of mouse leukemia viruses can be carried in the air and that inhalation, by mice, of contaminated air results in infection and leukemia. To protect animals, laboratory workers, and other people from possible disease by viruses known or suspected of causing leukemia, air filters and systems have been developed with a 99.99% efficiency.

This is important because it alerts us to the need for protection of laboratory workers and animals from potential infection with uncommonly strong preparations of viruses studied in the laboratory. The concurrent finding that low doses of virus (roughly equivalent to those found in nature) were not transmitted through air is also important because it provides additional indirect evidence that human leukemia probably is not contagious.

I 1

DATE: February 28, 1967

SPECIAL VIRUS LEUKEMIA PROGRAM

Segment Chairman's Combined Summary

of

Triannual (Annual) Progress Reports

Period Covered: October 1, 1966 through January 31, 1967

Program Segment: Developmental Research

Contracts Included in Combined Summary:

1. Germfree Life Research Center (PH43-65-95) - Germfree Life and Oncogenesis.
2. Chas. Pfizer & Co., Inc. (PH43-66-98) - Development of Virus Tumor Test Systems.
3. Children's Hospital of Philadelphia (PH43-66-477) - Interference and Immunofluorescence Studies with Cell Lines Derived from Leukemia and Lymphoma.
4. Germfree Products, Inc. (PH43-64-533) - Production of Murine Leukemia Seed Stocks.
5. Health Research, Inc. (PH43-63-593) - Electron Microscopy for Studies of Viruses in Human Leukemic Cells.
6. M. D. Anderson Hospital and Tumor Institute (PH43-65-604) - Studies on the relationship of Viruses to Murine Leukemia and Lymphoma.
7. Health Research, Inc. (PH43-65-616) - Development of a Large Volume of Leukemic Cells.
8. University of Texas Dental Branch (PH43-65-628) - Human Leukemia-Lymphoma Study.
9. The Regents of the University of Michigan (PH43-65-639) - Continue Research Relating to Etiology of Human Leukemia.
10. Bionetics Research Laboratories, Inc. (PH43-67-661) - Investigation of the Carcinogenic Activity of Selected Virus Preparations in Newborn Monkeys.

11. Microbiological Associates, Inc. (PH43-67-697) - Evaluation of Human Tumors in Newborn Mice and Bioassay of Animal Tumor Virus.

12. Rutgers University (PH43-65-1039) - Conduct immuno-chemical and antigenic studies of known and candidate leukemia viruses.

Segment Chairman: Robert A. Manaker
Robert A. Manaker, Ph.D.

Segment Vice Chairman: C. J. Dalton
A. J. Dalton, Ph.D.

Murine leukemia virus studies continue at a reduced level. Production of the Rauscher and Moloney strains from mouse plasma at the John L. Smith Memorial for Cancer Research, Chas. Pfizer and Co., Inc., remains unchanged. However, tissue culture mouse virus production was discontinued with 12 liters of Moloney virus and 2 liters of Rauscher virus in storage. The program at Germ Free Products, Inc. to produce mouse leukemia viruses in SPF mice for immunological investigations provided adequate amounts of Friend, Rauscher and Moloney viruses. 30 percent of requirements for Breyer-Moloney, Manaker and Buffet viruses, and some rat passaged Rauscher and Moloney viruses.

Quantitation of Rauscher strain virus by application of the latex particle method showed that virus concentrations of 2-5 X 10^7 virus particles per ml applied to E.M. screen grids in accordance with routine negative staining procedures resulted in an average distribution of one particle per grid square. This provides a basis for ready estimation of virus content of samples. Virus assay has also been continued in mice to quantitate leukemogenic activity. During the past quarter, 75 bioassays were completed and 60 new assays were initiated.

Immunological studies showed the Rauscher and Moloney strains of virus chemically coupled to sheep red blood cells as a carrier were highly immunogenic in mice and rabbits. The viruses were readily inactivated. Common antigenic determinants among the Rauscher, Moloney, Friend and Gross virus strains were demonstrated by immunodiffusion. Ether treatment of the Rauscher virus strain yielded RNAase-sensitive nucleoids infective for tissue cultured cells and newborn mice but not reactive in neutralization or complement fixation tests.

A normal mouse bone marrow cell line, designated V9 and maintained in long-term culture, was shown to be susceptible to infection by the Rauscher, Friend, Moloney and Gross virus strains. Uninfected V9 cells had undergone a change to malignancy demonstrated by the formation of localized tumors following infection into mice. When V9 cells were infected with Rauscher virus, their ability to grow as a localized tumor was lost. The phenomenon is under study.

The V9 culture inoculated with Rauscher virus-infected mouse tissue and designated V10 has been used for virus production. As previously observed with virus produced by the V5 culture, virus yielded by culture V10 lost leukemogenic activity. The change in this property was rapid at about the 60th serial subculture of the cell line. The V10 virus is not as effective as is V5 virus in inducing mouse resistance to subsequent challenge with leukemogenic Rauscher plasma virus and may, therefore, differ from it in some respects.

The effort to detect, isolate and characterize viruses which may be of etiological significance in human leukemia has expanded during the last quarter. Six new cell lines developed at Chas. Pfizer & Co., Inc., from

fifty-four blood or other tissue specimens, all carried a herpes-type virus morphologically similar to that found in cultured Burkitt lymphoma cells. At Health Research, Inc., ninety-five new white blood cell lines were established from fifty-five donors. About one third contained the herpes-type virus. Sixteen of the lines were started from eleven non-leukemic donors. Three of these lines carry the herpes-type virus.

At the University of Michigan, activity is directed to the search for C-type particles using four established human cell lines as host cultures for virus. Electron microscope monitoring of inoculated cultures disclosed type C particles in a few cultures which could not be verified as virus in the absence of observation of budding. Bone marrow specimens from 228 leukemic and fifty-three non-leukemic children as well as eighty-six leukemic and fifty-six non-leukemic adults were tested in tissue cultures. Some viruses were isolated and identified as adeno-, herpes, or parainfluenza viruses. One isolate is as yet unidentified. Direct examination by electron microscopy of bone marrows or sedimented plasma specimens detected no virus. Direct cultivation of bone marrow, lymph node, and white blood cells from patients with and without leukemia and subsequent exposure of the four established human cell lines to these cells was conducted. Unequivocal cytopathogenic changes were observed in some cultures so exposed to cells from leukemic donors. Mycoplasma could not be implicated. Direct cultivation of 181 specimens of tissue from patients with leukemia was also carried out at the M. D. Anderson Hospital and Tumor Institute. Fifteen cultures remained viable after 100 days of incubation. Two of four bone marrow cultures cultivated for two months contained particles resembling the C-type. Direct electron microscope examination disclosed particles resembling C-type in six lymph node specimens from six patients. This success may be ascribed to the exhaustive search conducted in this laboratory.

The production of herpes-type virus from a culture of the African Burkitt lymphoma has been increased at Chas. Pfizer and Co., Inc., by a substantial increase in the virus content of the culture. Other Pfizer cell lines reportedly produce comparatively high yields of herpes-type viruses and promise to be useful for comparative studies. Techniques practiced at Health Research, Inc., involving modification of cultural conditions have also effectively raised virus yield in the African lymphoma culture.

The mixed hemaglutination test is being applied at M. D. Anderson Hospital and Tumor Institute to sera from patients with leukemia in the expectation that some will contain antibodies against candidate viruses or tumor antigen. These sera would provide tumor specific reagents for future studies.

Immunofluorescence (FA) tests conducted at the Children's Hospital of Philadelphia using selected human sera showed that positive fluorescence of cultured cells from patients with Burkitt lymphoma, leukemia and some

normal individuals was accompanied by intracellular herpes-type virus detectable by electron microscopy, strongly suggesting that the fluorescence was specific for viral antigen. The herpes-type particles in the P3J ("Jiyoye") and EB3 cell lines could not be identified with herpes simplex, cytomegalo or varicella virus. Indirect FA tests against P3J and EB3 cells with sera from twenty chimpanzees, forty-six baboons, nine rhesus monkeys, ten normal and lymphomatous cattle, seventy-one normal dogs and thirty-one lymphomatous dogs were negative. Weakly positive FA tests were obtained with sera from four primates that had received injections of Burkitt lymphoma materials. The Fink/Malmgren FA technique was used at the University of Michigan to check 100 bone marrow specimens. Marrow cells from eighty percent of patients with acute lymphatic leukemia and thirty-three percent of patients in remission were positive while marrows from control patients were negative. A strongly positive marrow was obtained from a patient with prostatic carcinoma.

Human sera from different donors and suitably absorbed rabbit antisera to the herpes-type particles associated with lymphoma cultures were used at Chas. Pfizer and Co., Inc. to demonstrate coating of naked herpes-type virus particles from different cell lines. Coating of herpes simplex virus by these sera was not observed. The sera from cancer patients were more strongly reactive than those from normal individuals.

The primate testing program provided 133 live births with only eleven percent mortality during the reporting period. At present 879 breeding animals of nine species are on hand. 755 animals are in holding. Ninety-five newborn animals were inoculated.

Examination of oral and anal swabs of newborn primates for agents cytopathogenic for monkey kidney culture yielded seventy-one adenoviruses, thirty-six enteroviruses, and three unidentified agents. A colony-wide survey using immunofluorescence methods is underway to specifically identify Vibrio species isolated. Vibrio fetus may be responsible for abortions and infant deaths.

Studies initiated include the inoculation of two immature chimpanzees, one with the AL-2 cultured American lymphoma cells and extracted virus and one with F132 cells and virus. The latter culture was originated from a patient with reticulum cell sarcoma. Cultures started from the peripheral blood of these animals three days after inoculation of the viable human cells contained cells morphologically similar to those inoculated. The herpes-type virus was observed in these cells insuring that opportunity to infect the primate host with the agent was maximal.

In another study, nineteen newborn monkeys were inoculated with freshly-drawn whole blood from patients at the NIH Clinical Center. A special project in progress involves induction of malaria in the recipient prior to inoculation with Burkitt virus.

SPECIAL VIRUS-LEUKEMIA PROGRAM

SEGMENT CHAIRMAN'S COMBINED SUMMARY
of
TRIANNUAL (ANNUAL) PROGRESS REPORTS

Period Covered: October 1, 1966 - January 31, 1967

Program Segment: TESTING AND MONITORING

Contracts Included in Combined Summary:

1. Baltimore Biological Laboratories (PH43-62-839) Development of Fluorescent Antibody Reagents for Selected Mycoplasma Strains

2. Baltimore Biological Laboratories (PH43-63-1161) Fluorescent Antibody Studies of Mouse Viruses

3. Baylor University (PH43-63-590) -- Studies on Viruses as Related to Cancer with Emphasis on Leukemia, and Continuation of the Testing Program in Primates

4. Chicago Park District (PH43-65-1017) -- Marmoset Breeding Colony

5. Flow Laboratories, Inc. (PH43-65-634) -- Virus Laboratory for Cancer Research

6. Wistar Institute (PH43-65-1028) -- Human Leukemia-Virus Test System: Problem Survey

7. Wistar Institute (PH43-65-1002) -- Study of Role of Mycoplasma Isolated from Human Leukemia Specimens

Segment Chairman

Segment Vice-Chairman Mary C. Finkle

The need for monitoring for Mycoplasma present as contaminants -- either airborne or from contamination of a tissue culture ingredient -- has been met in two ways: a) by establishing at the Wistar Institute, under the direction of Dr. Leonard Hayflick, a laboratory diagnostic service to which any SVLP investigator may send specimens for screening and identification; and b) by the preparation of immunofluorescent reagents for thirteen strains of Mycoplasma often found as contaminants. These reagents have been produced by the Baltimore Biological Co. and distributed from there to various investigators for use within their own laboratories. During this terminal contract year the antisera are being packaged for storage and distribution from the NCI.

A comprehensive viral diagnostic laboratory is being established at Flow Laboratories under the direction of Dr. James Duff. Reagents are being prepared for identification of viruses associated with diseases of man and other animals. It is planned that appropriate reagents will be added to the laboratories' armamentarium to increase and broaden its scope and capabilities. -- Antiserums prepared in other laboratories against the "C" type and herpes-type viruses found in material from human leukemia/lymphoma patients have been tested at this facility for neutralization and CF reactivity against several recognized human and other animal viruses including members of the enterovirus, myxovirus and herpesvirus groups. No significantly positive results have been obtained to date.

An immunofluorescent plaque neutralization technique for lymphocytic choriomeningitis virus has been developed by Dr. R. Wilsnack at the Baltimore Biological Laboratory.

A project to begin in July, 1967 is designed to test the reliability of different serological techniques in differentiating between leukemic and normal cells, or between cells containing the Burkitt-associated virus, and those not containing the virus. The most reliable technique will be selected for a proposed sero-epidemiologic survey of human populations. Dr. Benyesh-Melnick, of the Baylor University contract, has developed one of the techniques to be tested.

SPECIAL VIRUS-LEUKEMIA PROGRAM

SEGMENT CHAIRMAN'S COMBINED SUMMARY
of
TRIANNUAL (ANNUAL) PROGRESS REPORTS

Period Covered: October 1, 1966 - January 31, 1967

Program Segment: RESOURCES AND LOGISTICS

Contracts Included in Combined Summary:

1. California, University of (PH43-63-13)
   Mammalian Cell Culture Laboratory

2. Connecticut, University of (PH43-62-505)
   Establishment of a Specific Pathogen Free Flock of White Leghorn Chickens

3. Flow Laboratories, Inc. (PH43-65-1012)
   Low Temperature Specimen Repository

4. Hazleton Laboratories, Inc. (PH43-65-635)
   Rauscher Leukemia Virus Production

5. Hospital For Sick Children (PH43-65-97)
   Procurement of Plasma from Acute Leukemic Patients

6. Hyland Laboratories (PH43-66-894)
   Facility for the Procurement and Distribution of Sterile, Unfiltered Fetal Calf Serum

7. Hyland Laboratories (PH43-66-539)
   Serum Testing Laboratory

8. Louisville, University of (PH43-66-902)
   Preparation of Simian Foamy Virus Reagents and Antisera

9. Makerere University College (PH43-67-47)
   Epidemiologic Study of Burkitt's Lymphoma

10. Massachusetts General Hospital (PH43-65-1034)
    Preservation of Platelets and Leukocytes by Freezing

11. Melpar, Inc. (PH43-65-641)
    Human Embryonic Tissue and Cell Biology Facility

12. Melpar, Inc. (PH43-65-1033)
    Moloney Virus Production

13. Montreal Children's Hospital, The (PH43-65-1020) III-2
    Procurement of Plasma from Acute Leukemic Patients

14. Notre Dame, University of (PH43-65-1018)
    Development of Leukemia Virus-Free Germfree Mouse Colony

15. Southwest Foundation for Research and Education (PH43-66-950)
    The Production of Simian Virus and Homologous Antiserum

16. Tulane University (PH43-64-881)
    Evaluation of Tree Shrew as Tumor-Virus Research Animal

17. University Laboratories, Inc. (PH43-66-1133)
    Rous Sarcoma Virus Production

Segment Chairman R. Emerson

Two contracts, Hospital for Sick Children, Toronto, and Montreal Childrens Hospital, which provide clinical specimens from untreated acute leukemia cases were requested to supply serums rather than the previously supplied plasma. Output from these sources will form part of the serum collection necessary for a comparative study of new immunological tests.

Contributing to the serum collection will be the Makerere University contract which is providing sera from Burkitt lymphoma patients, their family, and control sera. A combined epidemiological and descriptive study of Burkitt lymphoma in Uganda, this contract will serve as a nucleus for a coordinated program to study that lymphoma as a disease problem and not simply a source of material useful to the laboratory. In FY-68 for example, the NCI Chemotherapy Area will send a chemotherapy team to Uganda to uncover, describe, study and treat the disease as it occurs in that area.

Continuing study of hematopoetic tissues and peripheral leukocytes in tissue culture in a search for viral agents is still dependent upon a supply of fresh tissue. Substantial progress is being made under the Massachusetts General Hospital contract (PH43-65-1034) to preserve human leukocytes and platelets. Platelet preservation techniques described last year are being evaluated clinically and by chromium tagging techniques. This year, small amounts of leukocytes are being successfully frozen with ethylene glycol as a protective agent. Further success in this area will provide for banking of leukocyte specimens for future work.

A contract with Melpar for a Human Embryonic Tissue and Cell Biology Facility is also oriented toward the cryobiological field in the processing of human perinatal kidney and lung tissues which are used extensively in virus isolation and assay procedures by contractors and collaborating scientists within the SVLP and virus cancer programs of NCI. Frozen, characterized and concentrated suspensions of embryonic cells ensure a continuing and dependable source of material for pertinent programs.

A Melpar contract as well as contracts with Hazelton Laboratories and Mason Research Institute supplied large quantities of Rauscher and Moloney murine leukemia virus concentrates to the SVLP. A product, extensively monitored for extraneous viruses, is available through the utilization of the VCB virus testing service. The mice used in virus production are also routinely tested.

The problem of latent viruses in laboratory animals continues to stimulate further research and development directed toward obtaining virus-free and virus-defined genetic stocks. The University of Connecticut contract has provided a source of chickens and eggs free from most poultry pathogens. The problem of Marek's disease has proven very difficult but hope of a possible solution has been raised by the generation of a small nucleus stock so far found to be free of the disease. The eggs in excess of breeding needs from this project have been supplied not only to virus cancer investigators but also to the Division of Biologics Standards for use in measles vaccine assays.

At the Lobund Laboratory, University of Notre Dame, further attempts have been made to find strains of mice free from latent murine leukemia viruses. All domestic strains of laboratory mice examined to date have shown evidence of leukemia-like virus in their thymic tissues. Viruses have not been detected in a strain of wild mice and a strain of laboratory mice, XIII, from France. Work on these will continue since the presence of latent leukemia viruses increases the difficulty in interpretation of the results of every experiment using mice as hosts for human or animal leukemia experiments.

Problems of latent agents and extraneous microbial contamination are not confined to laboratory animals but also include the cell cultures derived from animals or man.

The Cell Culture Collection Committee established by the NCI in 1960 has effectively dealt with these problems since its inception and now continues under sponsorship of the American Type Culture Collection to add new characterized cell lines to its repository. The University of California - Naval Biological Laboratory contract has been a cornerstone of this program by supplying non-primate mammalian cell lines to the scientific community. Development of needed bovine cell cultures was successfully undertaken for the SVLP Special Animal Leukemia Ecology Studies working segment and other cell lines from domestic and wild animals continue to build up our armamentarium of reagents with which to do comparative virology.

This contract has also provided research support to another Resources and Logistics program, that of defining and improving the widely-used fetal calf serum.

Study of individual samples at the Naval Biological Laboratory established that seven of thirty fetal calf serums contained significant amounts of gamma globulin, and that two of these seven serums contained significant amounts of specific antibody against bovine virus diarrhea. Likewise, testing of fetal calf serum in Dr. Dalton's laboratory and in another laboratory of the Cell Culture Committee, Dr. Cruickshank's, revealed that some fetal calf serums contained virus-like particles in the ultracentrifuged sediments examined by electron microscopy. The biological activity of these particles remains to be established but does raise the question of fetal calf serums contribution of latent viruses to cell culture systems.

These findings support the belief that continual refinement and characterization of biologically derived materials used in research is a practical necessity. In order to do this, two contracts with Hyland Laboratories (PH43-66-539) and (PH43-66-894) provided for production and testing capabilities for fetal calf serum. The aims of this program have been to develop criteria and specifications for an acceptable and reliable serum useful for both cell culture and virological studies. Since a tissue culture virological laboratory will use from 50-100 liters or more of this material per year it is important that increasingly stringent quality standards be evolved.

Finally, contracts for production of simian virus reagents have provided additional tools for detection and identification of this important group of viruses. Simian virus reagent production should be completed by 1969 and will become a basic resource for primate investigations.

IV-1

DATE: March 1, 1967

SPECIAL VIRUS-LEUKEMIA PROGRAM

Segment Chairman's Summary
of
TRIANNUAL (ANNUAL) PROGRESS REPORT

Period Covered: October 1, 1966 - January 31, 1967

Program Segment: Epidemiology

Contractor: National Center for Health Statistics (D.C.)

Contract Number: FS-(66)-35

Title: Deaths Certificates on Childhood Cancer, 1960-1964

Contractor's Project Director: Dr. Robert D. Grove

Project Officer (NCI): Robert W. Miller, M. D.

Objectives: To obtain death certificates for all U. S. children under 15 years of age who have died of cancer, 1960-1964. This registry will be used to determine 1) aggregation of cancers in twins or other sibs, 2) geographic clustering by dates of birth or death, 3) the association of cancer with congenital defects, and 4) the frequencies of specific cancers not separately listed in published mortality statistics; and 5) the registry will also be used to match against names of children believed to be at unusual risk of cancer; e.g., those given certain vaccines in the past.

Date Contract Initiated: Fy-1967

Current Annual Level: $8,500

Total Funding Committed to Date: $8,500

Progress: Work completed. About 22,000 copies of death certificates have been received.

Problems: None

Projections: Copies of corresponding birth certificates will be sought from each State. The data from the birth and death certificates will be abstracted and card-punched; lists and tables will be made to obtain the objectives described above.

The file will be up-dated at two-year intervals by obtaining childhood death certificates since the previous cut-off date.

IV-3
DATE: March 1, 1967

SPECIAL VIRUS-LEUKEMIA PROGRAM

Segment Chairman's Summary
of
TRIANNUAL (ANNUAL) PROGRESS REPORT

Period Covered: October 1, 1966 - January 31, 1967

Program Segment: Epidemiology

Contractor: National Academy of Sciences-National Research Council

Contract Number: 43-64-44, Task Order No. 23

Title: Investigations of Viral and Other Factors in Cancer Etiology in Army Veterans

Contractor's Project Director: Mr. Seymour Jablon

Project Officer (NCI): Robert W. Miller, M. D.

Objectives: 1) To study the cancer experience of 23,149 soldiers following World War II in relation to diagnoses made during military service and suspected to increase or decrease cancer occurrence; e.g., infectious mononucleosis, infectious hepatitis and herpetic infections.

2) To study about 3,200 veterans who developed cancer, 1950-1954, to determine if frequencies of certain events during military service, 1942-1944, exceed those for a matched control group; e. g., blood transfusion and yellow-fever vaccination.

Date Contract Initiated: June 1, 1965

Current Annual Level: $34,900

Total Funding Committed to Date: $65,900

Progress: Excellent. Progress reports reveal that study is on schedule and going as planned.

Problems: None.

Projections: Should finish on time.

SPECIAL VIRUS-LEUKEMIA PROGRAM

SEGMENT CHAIRMAN'S COMBINED SUMMARY
of
TRIANNUAL (ANNUAL) PROGRESS REPORTS

Period Covered: October 1, 1966 - January 31, 1967

Program Segment: SPECIAL ANIMAL LEUKEMIA ECOLOGY STUDIES

Contracts Included in Combined Summary:

1. University of California (PHS43-65-609) - - Bovine Leukemia Transmission Studies.

2. Cornell University (PH43-65-620) - - Research on Susceptibility of Cat to Viral Leukemogenesis.

3. Michigan State University (PH43-65-100) - - Human/Canine Leukemic Transmission Trials: Etiology of Canine Leukemia.

4. University of Pennsylvania (PH43-65-1013) - - Research in Experimental and Natural Transmission of Bovine Leukemia.

5. Research Foundation of the State University of New York (PH43-65-605) - - Comparative Studies on Structure of Known Viruses and Particles Observed in Bovine Tissues.

6. Einstein Medical College, New York (PH43-65-612) - - Research on Immunological Factors in Susceptibility to Murine Leukemia Viruses.

7. Taft Sanitary Engineering Center, Cincinnati, Ohio (PHS-VCL-30) Studies on Thermal Inactivation of Viruses in Milk and Milk Products.

8. Institute for Medical Research, Camden, New Jersey (PH43-65-65) To Conduct and Etiological Study on Bovine Leukemia.

9. University of Minnesota (PH43-65-606 - - Research on Susceptibility of Cows to Known Tumor Viruses.

10. University of North Dakota (PH43-66-8) - - Insects as Possible Experimental and Natural Vectors of Murine and Avian Tumor Viruses.

Segment Chairman _____

Segment Vice-Chairman _____

Research efforts conducted within the Special Animal Leukemia Ecology Segment of the Special Virus-Leukemia Program are concerned with determining the etiology of leukemia as it occurs in certain domestic animals. In addition, a primary effort of this Segment is to determine the relationship, if any, of animal leukemias to lymphoma and/or leukemia in man. Studies within the SALES program may be grouped as follows:

RESEARCH IN BOVINE LEUKEMIA. Dr. Robert Marshak, University of Pennsylvania, is maintaining an "experimental" bovine lymphosarcoma by whole-cell transplant of the neoplasm in irradiated calves. The role of known carcinogenes in the induction of bovine leukemia is being studied. It is hoped that these materials will serve as virus as virus source tissues. Reciprocal foster nursing experiments have begun to clarify the relationship between ingestion of "viremic" milk and the occurance of bovine leukemia or lymphocytosis. This researcher has established in tissue culture five cell lines derived from leuekemic cows. These lines are in stationary and/or spinner cultures. Three lines are of buffy coat origin; two lines are of lymph node origin. The cells are lymphocytic in appearance and aneuploid. The lines are to be checked for the presence of virus particles by electron microscopy.

Dr. Ray Dutcher, Institute for Medical Research, Camden, New Jersey is collaborating with Dr. Marshak in the latter study. In addition, this researcher has established and partially characterized a continuous kidney line from a leukemic cow. It is believed that this line will contribute to standardization of virus interference studies and quantitation of interferon-like activity. Dr. Dutcher is conducting electron microscopic surveys of bovine mammary gland tissue. Virus particles have been found in a certain number of these specimens. The particles were usually found in ducts and/or vesicles within the cell.

Dr. Charles Burger, Upstate Medical Center, State University of New York, is also collaborating with this group. He has accomplished separation of Type C particles from the milk of leukemic cows. These have been isolated in large

enough quantities to permit definitive characterization of the particles. These virus fractions have been extracted for nucleic acid and the data indicated that RNA is present which is presumably of the single-stranded nature. This researcher is also collaborating with Dr. George Moore's group, Roswell Park. He has examined large volumes of tissue culture fluid from Burkitt cell lines. Herpes-type particles have been recovered in relatively large numbers. Chemical data on these fractions have been obtained indicating a preponderance of DNA.

Dr. Gordon Theilen, University of California, has been able to establish a good correlation between onset, degree and persistence of lymphocytosis in cows with concentration of inoculated normal and/or leukemic bovine buffy coat materials. Although none of the animals inoculated with tumor tissues have developed leukemia, many have extreme blood dyscrasis. Electron microscopy of pellets derived from leukemic plasma of inoculated animals have revealed particles, some of which resemble the Type C particle. Cytogenetic studies conducted by this investigator on animals which received tumor whole-cell material indicate a chromosomal structural change related to persistant lymphocytosis. In some animals the number of cells with polyploidy was increased above the normal.

Dr. D. K. Sorenson, University of Minnesota, has inoculated 26 calves with selected murine leukemia viruses. Response of the animals to the viruses is being followed through hematological, electron microscopic, and seriological studies. Intrauterine fetal inoculation of the leukemia viruses is also being studied in 48 cows bred by artificial insemination. Both the Moloney Leukemia Virus and Rauscher Leukemia Virus appear to infect a continuous line of bovine endocardial cells as determined by direct fluorescent antibody techniques.

Dr. R. B. Read, Taft Sanitary Engineering Center, Cinncinati, Ohio, has developed thermal destruction rate curves for the following viruses: Herpes-simplex Adeno-12, Sv-40, Reo 1, Rauscher and Moloney Leukemia Viruses. A tissue sample from a human leukemia patient maintained "in vitro" has yielded plaque forming agents. This material will be studied by the National Cancer Institute staff for

viral characterization. Engineering studies are progressing which will result in the development of more effective pasteurization procedures. During the next year, virus inactivation in sewage will be studied in collaboration with the Biohazards Segment of the Special Virus-Leukemia Program.

RESEARCH IN CANINE LEUKEMIA. The principal effort in this area is concentrated at the Michigan State University, Lansing, Michigan under the direction of Dr. Gabel Conner. Here twenty types of candidate leukemogenic materials, both human and animal derived, have been inoculated into 226 Caesarean-derived neonatal Beagle dogs housed in isolation quarters. Eight percent of the dogs which have received leukemic whole-cell or cell-free dog material have developed malignant tumors. Some dogs, including those which received human inocula developed a lymphadenopathy. Hetero-species induction of malignant lymphosarcoma (reticulum cell sarcoma) occurred in one of eleven animals which received Moloney sarcoma virus. Electron microscopic studies conducted by these investigators have describe intracytoplasmic crystalline arrays of particles, 18 to 22 mu in diameter in malignant tissue of 100% of the induced and 90% of the spontaneous cases of canine lymphoma. Antigenic studies using immunofluorescence have shown that human gamma globulin reacts positively with Burkitt cells. Serologic studies on dogs with lymphosarcoma have shown the presence of specific complement fixing antibody which reacts to human mumps antigen. The host cell-virus infectivity spectrum has been determined for canine thymus cells using certain candidate viruses.

RESEARCH IN FELINE LEUKEMIA. Dr. Charles Rickard, Cornell University has been able to transmit lymphosarcoma using cell-free preparations from an American cat to three new-born animals of the same species. Type C virus particle have been found budding from the plasma membranes of one cat with spontaneous lymphocytic leukemia. They appear morphologically different from the known cat viruses. A tissue culture cell-line has been established from a cat neurofibrosarcoma.

INSECT VECTOR STUDIES. Dr. Robert Fischer, University of North Dakota, has shown that selected blood-sucking insects can transmit the Twiehaus virus from infected chickens to normal hosts. The virus is viable within the insect for at least 72 hours. The blood-sucking insect, Triatoma infestans as well as Bedbugs, can transmit Rauscher Leukemia Virus from viremic mice to normal hosts of the same species. This virus remains active in the infected insects for an extended period of time. Studies will be extended to include the Moloney Sarcoma Virus because of the rapid bio-assay methods now available for this agent.

GENETIC STUDIES. Studies on genetic and immunological factors which play a role in the susceptibility of animals to tumor viruses are being conducted by Dr. Frank Lilly, Einstein Medical College. In the Friend virus leukemia system there are indications that the H-2 type influences the ability of animals to respond immunologically to the virus antigens. Experiments to study levels of antibody production in relation to H-2 type are now in progress. In both the Friend and Gross leukemia virus systems other genes effect susceptibility or resistance. The differentiation according to host strain specificity of two strains of Friend Virus has been completed.

DATE: March 1, 1967

SPECIAL VIRUS-LEUKEMIA PROGRAM

Segment Chairman's Combined Summary
of
TRIANNUAL PROGRESS REPORTS

Period Covered: October 1, 1966 - January 31, 1967

Program Segment: BIOHAZARDS CONTROL AND CONTAINMENT

Contracts Included in Combined Summary:

1. The Dow Chemical Company (PH43-65-1045)
   Research and Development on Biohazards Control and Containment Facilities

2. U.S. Department of the Army, Ft. Detrick (NCI - FS - (66) - 37)
   Evaluation of Filters with Sub-Micron Aerosols

3. The Ohio State University Research Foundation (PH43-65-1001)
   Hazards of Experimental Leukemia Research.

4. University of Minnesota (PH43-65-999)
   Development of an "Open Isolation" System for the Care of Low Resistance Patients.

Segment Chairman_____

Segment Vice-Chairman_____

Under the research and development contract on biohazards control and containment, several significant areas have been investigated.

Automatic Watering Devices: Of the three commercially available valves tested to date, only one valve prevented passage of Pseudomonas aeruginosa to uninoculated mice for periods up to seven days. More extensive tests are planned.

Mobile Virus Laboratory: Construction of the trailer and laboratory components is on schedule and will be completed during remainder of FY 1967.

Simulated Laboratory and Safety Cabinet Evaluations: Smoke evaluation has been completed and preliminary studies of particle size distribution of Serratia marcescens from various virus laboratory accidents have been completed.

Prototype Laboratories: Preliminary designs for a virus concentration laboratory and safety monitoring laboratory have been submitted to NCI for review and approval.

Biohazards Warning Symbol: Decals, use-standards and posters are currently being evaluated by NCI laboratories and selected contract facilities. The symbol has been adopted by the Agricultural Research Service, USDA.

The studies which are being conducted with ultrahigh-efficiency filters (HEPA) challenged with T_1 bacteriophage (0.1 micron NMD) will provide additional information concerning the correct, safe application of these filters in laminar flow safety cabinets and rooms for virus research and patient care. In addition, studies are in progress to determine aerosol transmission in four viral-host systems, i.e., 1) newborn hamsters and type 12 adenovirus 2) BALB/C mice and Rauscher virus, 3) yearling monkeys and Yaba virus and 4) newborn marmosets or monkeys and Rous-sarcoma virus. Results of these

studies thus far indicate that natural transmission of oncogenic viruses, particularly Rauscher murine leukemia virus, by aerosols is a definite possibility.

The evaluation of the concept of horizontal laminar flow as a patient isolation system has shown that the concept is feasible. Additional efforts will be expended to define design criteria and operational procedures for such a facility.

SVLP CONTRACTORS

1. Athey, W.: BALB/c Mouse Colony. Microbiological Associates, Inc., Walkersville, Maryland, PH43-66-914, Program Management.

2. Bernstein, E.: Rous Sarcoma Virus Production. University Laboratories, Highland Park, New Jersey, PH43-66-1133, Resources and Logistics Program Segment.

3. Biddle, J. L.: Preparation of Feline Virus Reagents and Antisera. Dow Chemical Company (Pitman-Moore Division), Zionsville, Indiana, PH43-67-657, Resources and Logistics Program Segment.

4. Burger C. L.: Comparative Studies on Structure of Known Tumor Viruses and Particles Observed in Bovine Tissues. Research Foundation of State University of New York, Syracuse, New York, PH43-65-605, SALES Program Segment.

5. Carski, T. R.: Development of Fluorescent Antibody Tests for Myco plasma. Baltimore Biological Lab., Inc., Baltimore, Maryland, PH43-62-839, Testing and Monitoring Program Segment.

6. Cascardo, M.: Primate Viral Diagnostic and Reference Reagents Lab. Human Leukemia Virus Lab. Flow Laboratories, Rockville, Maryland, PH43-67-1135, Testing and Monitoring Program Segment.

7. Conner, G.: Human/Canine Leukemic Transmission Trials: Etiology of Canine Leukemia. Michigan State University, East Lansing, Michigan, PH43-65-100, SALES Program Segment.

8. Darte, J. M.: Human Leukemia and Normal Tissue Collection and Preservation. Hospital for Sick Children, Toronto, Canada, PH43-65-97, Resources and Logistics Program Segment.

9. Decker, H. M.: Study of Filters with Submicron Aerosols. Department of the Army, Ft. Detrick, Frederick, Maryland, FS-37, Biohazards Control and Containment Program Segment.

10. Defendi, V.: Problem Survey of Human Leukemia Virus Test Systems. Wistar Institute, Philadelphia, Pennsylvania, PH43-65-1028, Testing and Monitoring Program Segment.

11. Denton, R. L.: Supply of Leukemic and Normal Blood Plasma Specimens. Montreal Children's Hospital, Montreal, Canada, PH43-65-1028, Testing and Monitoring Program Segment.

12. Doll, E. R.: Preparation of Antigens and Antisera for Equine Herpes Viruses, University of Kentucky, Louisville, Kentucky, PH43-67-1140, Testing and Monitoring Program Segment.

13. Dmochowski, L.: EM, Virological Tissue Culture and Immunofluorescence Studies of Human Leukemia/Lymphoma Tissues Mycoplasma Monitoring. University of Texas, Houston, Texas, PH43-65-604, Developmental Research Program Segment.

14. Dutcher, R.: Etiological Studies of Bovine Leukemia. South Jersey Medical Research Foundation, Camden, New Jersey, PH43-65-65. SALES Program Segment.

15. Edington, G. M.: Virological Studies of Burkitt Lymphoma Specimens. University of Ibadan, Ibadan Nigeria PH43-67-674, Resources and Logistics Program Segment.

16. Farrow, M.: Production of Murine Leukemia Virus Seed Stocks. Germfree Products Inc., St. Petersburg, Florida, PH43-64 533, Developmental Research Program Segment.

17. Fischer, R. G. Insects as Vectors of Tumor Viruses. University of North Dakota, Grand Forks North Dakota, PH43-66-8, SALES Program Segment.

18. Fisher L. E.: Marmoset Breeding Colony. Chicago Park District, Lincoln Park Zoo Chicago Illinois, PH43-65-1017 Testing and Monitoring Program Segment.

19. Gaeta, L. E.: Fetal Calf Serum Testing Laboratory. Hyland Laboratories Los Angeles, California, PH43-66-539, Resources and Logistics Program Segment.

20. Gaeta L. E.: Procurement of Fetal Calf Serum. Hyland Laboratories, Los Angeles, California, PH43 66-894 Resources and Logistics Program Segment.

21. Gillespie, J. H.: Preparation of Feline Virus Reagents and Antisera. Cornell University Ithaca New York, PH43-67-664, Resources and Logistics Program Segment.

22. Gori G. B.: Moloney Leukemia Virus Production. Melpar, Inc. Falls Church Virginia, PH43-65-1033. Resources and Logistics Program Segment.

23. Grace J. T.: EM Studies of Viruses in Leukemic Tissues and Plasma. Health Research Inc. Buffalo, New York PH43-63-593, Testing and Monitoring Program Segment.

24. Griesemer, R. A.: Aerosol Transmission of Oncogenic Viruses, Ohio State University, Columbus, Ohio, PH43-65-1001, Biohazards Control and Containment Program Segment.

25. Groupe, Vincent: Etiology of Marek's Disease. Rutgers, The State University, New Brunswick, New Jersey, PH43-67-1166, SALES Program Segment.

26. Grove, R. D.: Death Certificates on Childhood Cancer, 1960-1963. National Center for Health Statistics, PHS, Washington, D. C., PS-35, Epidemiology Program Segment.

27. Hayflick, L.: Study of Role of Mycoplasma Isolated from Human Leukemia Specimens. Wistar Institute, Philadelphia, Pennsylvania, PH43-65-1002, Testing and Monitoring Program Segment.

28. Henle, G.: Interference and Immunofluorescence Studies of Burkitt Lymphoma Cell. Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, PH43-66-477, Developmental Research Program Segment.

29. Honicker, F. A.: Low Temperature Specimen Repository. Flow Laboratories, Rockville, Maryland, PH43-65-1012, Resources and Logistics Program Segment.

30. Huggins, C.: Cyrogenic Preservation of Platelets and Leukocytes. Massachusetts General Hospital, Boston, Massachusetts, PH43-65-1034, Resources and Logistics Program Segment.

31. Jablon, S.: Investigations of Viral and Other Factors in Cancer Etiology in Army Veterans (Task Order No. 23). National Academy of Sciences, Washington, D. C., PH43-64-44, Epidemiology Program Segment.

32. Johnston, P. B.: Preparation of Simian Foamy Virus Reagents and Antisera. University of Louisville, Louisville, Kentucky, PH43-66-902, Resources and Logistics Program Segment.

33. Kalter, S. S.: Production of Simian Viruses and Homologous Antiserum. Southwest Foundation for Research and Education, San Antonio, Texas, PH43 66-950, Resources and Logistics Program Segment.

34. Levy, B.: Evaluations of Human Leukemia/Lymphoma Specimens in Marmosets. University of Texas (Dental Branch), Houston, Texas, PH43-65-628, Developmental Research Program Segment.

35. Lilly, F.: Research on Immunological Factors in Susceptibility to Murine Leukemia Viruses. Einstein Medical College, Bronx, New York, PH43 65-612, SALES Program Segment.

36. Luginbuhl, R.: SPF Colony of White Leghorn Chickens. University of Connecticut, Storrs, Connecticut, PH43-62-505, Resources and Logistics Program Segment.

37. Lutwama, J. S.: Collection of Burkitt African Lymphoma Tissue Specimens. Makerere University Medical School, Uganda, PH43-67-47, Resources and Logistics Program Segment.

38. Madin, S.: Mammalian Cell Culture Research, Propagation of Normal Bovine Cell Lines. University of California, Oakland, California, PH43-63-13, Resources and Logistics Program Segment.

39. Madin, S.: Facility for Univ. of California, (63-13) Cell Culture Research and Propagation. Naval Biological Laboratory, Oakland, California, PH-8, Resources and Logistics Program Segment.

40. Madison, R.: Bioassays of Animal and Human Tissues. Microbiological Associates, Inc., Walkersville, Maryland, PH43-67-697, Developmental Research Program Segment.

41. Marshak, R.: Establishment of Criteria for Normalcy vs. Leukemic Affection. University of Pennsylvania, Philadelphia, Pennsylvania PH43-65-629, SALES Program Segment.

42. Marshak, R.: Research on Experimental and Natural Transmission of Bovine Leukemia. University of Pennsylvania, Philadelphia, Pennsylvania, PH43-65-1013, SALES Program Segment.

43. Mattson, G.: Research and Development re Biohazards Containment Facilities. Dow Chemical Co., Midland, Michigan, PH43-65-1045 Biohazards Control and Containment Program Segment.

44. Meier, H.: Studies of Natural Occurrence of Murine Leukemia-Sarcoma Complex. The Jackson Laboratory, Bar Harbor, Maine, PH43-67-744 SALES Program Segment.

45. Melnick, J. L.: Research on Viruses in Human and Primate Leukemic Tissues and Plasma. Baylor University, Houston, Texas, PH43-65-590, Testing and Monitoring Program Segment.

46. Michaelson, G. S.: Develop Open Isolation System for Care of Low Resistance Patients. University of Minnesota, Minneapolis, Minnesota, PH43-65-999, Biohazards Control and Containment Program Segment.

47. Morrow, Richard L.: Collection of Burkitt African Lymphoma Specimens. Makerere University Medical School, Uganda, PH43-67-47, Resources and Logistics Program Segment.

48. Moore, G. E.: Development of Methodology for Large Scale In Vitro Propagation of Human Leukemic Cells. Health Research, Inc., Buffalo, New York PH43-65-616, Developmental Research Program Segment.

49. Murphy W. H.: Research on Virus Particles in Plasma and Tissue Culture Specimens. University of Michigan, Ann Arbor, Michigan. PH43-65-639. Developmental Research Program Segment.

50. Oleson J. J.: Development of Virus-Cancer Test Systems: Virus Production: Production of Human Virus Cancer Cell Lines. Charles Pfizer and Co., Inc. Maywood, New Jersey, PH43-66-98, Developmental Research Program Segment.

51. Palczuk, N. C.: Immunochemical and Antigenicity Studies. Rutgers University, New Brunswick, New Jersey, PH43-65-1039, Developmental Research Program Segment.

52. Pallotta, A. J.: Virus Cancer Studies in Primates. Bionetics Research Labs., Inc. Kensington, Maryland, PH43-62-412, Developmental Research Program Segment.

53. Pledger, R.: Rauscher Leukemia Virus Production. Hazleton Labs., Inc. Falls Church Virginia, PH43-65-635. Resources and Logistics Program Segment.

54. Pollard, M.: Development of Leukemia Virus-Free, Germfree Mouse Colony. University of Notre Dame South Bend Indiana, PH43-65-1018, Resources and Logistics Program Segment.

55. Read, R. B.: Studies on Thermal Inactivation of Viruses in Milk and Milk Products. PHS Bureau of State Services (Taft Sanitary Engineering Ctr.). Cincinnati Ohio VCL 30. SALES Program Segment.

56. Reyniers J. A.: Comparative Studies of Carcinogen Activated Viral Tumorigenesis in Germfree and Conventional Animals. Germfree Life Research Center, TAmpa Florida, PH43-65-95, Developmental Research Program Segment.

57. Rickard C.: Research on Susceptibility of Cat to Viral Leukemogenesis. Cornell University Ithaca, New York. PH43-65-620. SALES Program Segment.

58. Riopelle A. J.: Evaluation of Tree Shrew as Tumor Virus Research Animal. Tulane University, Covington Louisiana, PH43-64-881. Resources and Logistics Program Segment.

59. Sorensen D. K.: Research on Susceptibility of Cows to Known Tumor Viruses. University of Minnesota, St. Paul, Minnesota, PH43-65-606, SALES Program Segment.

60. Theilen G. H.: Bovine Leukemia Transmission Studies. University of California Davis California, PH43-65-609, SALES Program Segment.

61. Verna, J.: Human Embryonic Tissue and Cell Culture Facility: Cell Biology Research. Melpar Inc., Falls Church, Virginia, PH43-65-641, Resources and Logistics Program Segment.

62. Naravdekar, V. S.: Research on Host Cell Virus Interrelationships. Microbiological Associates, Inc., Bethesda, Maryland, PH43-66-887 Program Management Segment.

63. Wilsnack R.: Fluorescent Antibody Studies of Murine Viruses. Baltimore Biological Lab., Inc., Baltimore, Maryland, PH43-63-1161, Testing and Monitoring Program Segment.